FLIGHT METABOLIC RATE IN THE GLANVILLE FRITILLARY BUTTERFLY

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Academic dissertation

To be presented for public examination
with the permission of the Faculty of Biosciences of the University of Helsinki
in the Auditorium 1401 in Biocentre 2 (Viikinkaari 5) on December 4th 2009 at 12 o'clock noon.

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ISBN 978-952-92-6546-6 (paperback)
ISBN 978-952-10-5902-5 (PDF)
http://ethesis.helsinki.fi

Yliopistopaino
Helsinki 2009

CONTENTS

The thesis is based on the following articles, which are referred to in the text by their Roman numerals:

I Niitepõld, K. Genotype by temperature interactions in the metabolic rate of the Glanville fritillary butterfly. – *manuscript*

II Niitepõld K., Smith A.D., Osborne J.L., Reynolds D.R., Carreck N.L., Martin P.M., Marden J.H., Ovaskainen O. & Hanski I. 2009. Flight metabolic rate and Pgi genotype influence dispersal rate in the field. – *Ecology* 90(8): 2223–2232.

III Niitepõld K., Mattila A.L.K., Harrison P.J. & Hanski I. Flight metabolic capacity has contrasting effects on dispersal in the two sexes of the Glanville fritillary butterfly. – *manuscript*

IV Niitepõld K. & Hanski I. Positive association between flight metabolism and lifespan in a butterfly. – *manuscript*

CONTRIBUTIONS

Contribution table:

	I	II	III	IV
Original idea	KN	KN, IH, JM	KN, IH, PH	KN, IH
Study design	KN	KN, IH, JO, DR	KN, IH, ALM, PH	KN
Data collection	KN	KN, IH, AS, JO, DR, NC, APM, OO	KN, ALM, PH	KN
Analyses	KN	KN, IH, OO	KN, IH, ALM, PH	KN
Manuscript preparation	KN, IH	KN, IH	KN, IH, ALM, PH	KN, IH

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ABSTRACT

Dispersal is a highly important life history trait. In fragmented landscapes the long-term persistence of populations depends on dispersal. Evolution of dispersal is affected by costs and benefits and these may differ between different landscapes. This results in differences in the strength and direction of natural selection on dispersal in fragmented landscapes. Dispersal has been shown to be a non-random process that is associated with traits such as flight ability in insects. This thesis examines genetic and physiological traits affecting dispersal in the Glanville fritillary butterfly (*Melitaea cinxia*). Flight metabolic rate is a repeatable trait representing flight ability. Unlike in many vertebrates, resting metabolic rate cannot be used as a surrogate of maximum metabolic rate as no strong correlation between the two was found in the Glanville fritillary. Resting and flight metabolic rate are affected by environmental variables, most notably temperature. However, only flight metabolic rate has a strong genetic component. Molecular variation in the much-studied candidate locus phosphoglucose isomerase (*Pgi*), which encodes the glycolytic enzyme PGI, has an effect on carbohydrate metabolism in flight. This effect is temperature dependent: in low to moderate temperatures individuals with the heterozygous genotype at the single nucleotide polymorphism (SNP) AA111 have higher flight metabolic rate than the common homozygous genotype. At high temperatures the situation is reversed. This finding suggests that variation in enzyme properties is indeed translated to organismal performance. High-resolution data on individual female Glanville fritillaries moving freely in the field were recorded using harmonic radar. There was a strong positive correlation between flight metabolic rate and dispersal rate. Flight metabolic rate explained one third of the observed variation in the one-hour movement distance.A fine-scaled analysis of mobility showed that mobility peaked at intermediate ambient temperatures but the two common *Pgi* genotypes differed in their reaction norms to temperature. As with flight metabolic rate, heterozygotes at SNP AA111 were the most active genotype in low to moderate temperatures. The results show that molecular variation is associated with variation in dispersal rate through the link of flight physiology under the influence of environmental conditions. The evolutionary pressures for dispersal differ between males and females. The effect of flight metabolic rate on dispersal was examined in both sexes in field and laboratory conditions. The relationship between flight metabolic rate and dispersal rate in the field and flight duration in the laboratory were found to differ between the two sexes. In females the relationship was positive, but in males the longest distances and flight durations were recorded for individuals with low flight metabolic rate. These findings may reflect male investment in mate locating. Instead of dispersing, males with high flight metabolic rate may establish territories and follow a perching strategy when locating females and hence move less on the landscape level. Males with low metabolic rate may be forced to disperse due to low competitive success or may show adaptations to an alternative strategy: patrolling. In the light of life history trade-offs and the rate of living theory having high metabolic rate may carry a cost in the form of shortened lifespan. Experiments relating flight metabolic rate to longevity showed a clear correlation in the opposite direction: high flight metabolic rate was associated with long lifespan. This suggests that individuals with high metabolic rate do not pay an extra physiological cost for their high flight capacity, rather there are positive correlations between different measures of fitness. These results highlight the importance of condition.

SUMMARY

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1 Introduction

1.1 What is dispersal?

Dispersal is ubiquitous. Whether the focus is on individual life histories, gene flow among populations or global species ranges, dispersal plays a key role in shaping the process in question. Not surprisingly, dispersal has also drawn much attention from investigators with different backgrounds (Clobert et al. 2001, Bullock et al. 2002, Kokko and López-Sepulcre 2006, Ronce 2007, Nathan et al. 2008). Numerous definitions of dispersal exist, often related to the characteristics of particular study organisms and the spatial and temporal scales of interest. Passive seed dispersal in plants is a very different process from the behaviourally complex dispersal in mammals and birds. What is common, however, is the outcome: offspring will potentially reproduce at a different location than their mother.

Dispersal can occur at different life history stages. Especially in vertebrates dispersal is often categorised as natal or breeding dispersal (Clobert et al. 2001). Natal dispersal refers to dispersal before reproduction, while breeding dispersal occurs when an individual reproduces in two different spatial locations split by a period of dispersal. Both processes result in different generations reproducing at least partly in different spatial locations.

The outcome of dispersal may be clear, but the actual process of dispersal is very complex. The process of dispersal can be divided into three stages: emigration, transience and immigration (Clobert et al. 2001). Emigration involves leaving the current population, which may be the natal population. Emigration may be the easiest stage to study, although distinguishing emigration from mortality within the local population may often be problematic in field studies. The transience stage, also known as traversing or simply inter-patch movement, refers to moving in the habitat matrix. The matrix is not uniform and movements may be affected by the habitat type (Hein et al. 2003, Dover and Settele 2009). The last stage of dispersal, known as immigration, settlement or colonisation, is difficult to study for logistic reasons and is therefore not well known (Baguette and Van Dyck 2007). Immigration, too, is a multistage process starting from recognising the new habitat (Merckx and Van Dyck 2007), to the fitness of immigrants in their new population (Duckworth 2008) and to evolutionary processes following the settlement (Carroll and Dingle 1996). Often colonisation refers to immigration to a previously unoccupied habitat patch, thus leading to the establishment of a new local population.

The term 'migration' is often used interchangeably with 'dispersal'. For clarity it would however be useful to restrict the use of the word 'migration' to seasonal movements or (often two-way) movements driven by changes in environmental conditions and resource availability. In this case 'migration' refers to non-permanent displacement and 'dispersal' to permanent displacement.

1.2 Evolution of dispersal

There is a very extensive literature on the evolution of dispersal, and the key ultimate factors shaping dispersal in the evolutionary context appear to be well established (Clobert et al. 2001). The process is however a complex one and there may occur several strong selection pressures for and against dispersal (Heino and Hanski 2001, Ronce 2007). Several situations that tend to select for increased dispersal have been identified. For example, ephemeral habitat due to spatial or temporal variation in habitat quality is likely to increase dispersal propensity (Denno et al. 1996, Friedenberg 2003). Competition is seen as a potential driving force of dispersal (Lambin et al. 2001). The most acute form of competition is kin competition (Hamilton and May 1977), as it reduces the fitness of the focal individual both directly (due to competition with others) but also indirectly, via reduced fitness of relatives. Similarly, inbreeding

avoidance selects for dispersal and may co-occur with kin competition (Gandon 1999). Dispersal may also be beneficial as a means of evading predation or parasitism, although empirical evidence for this being a major factor in the evolution of dispersal is not particularly strong (Clobert et al. 2001).

The outcome of the evolution of dispersal balances the benefits gained by dispersing and the associated costs. The cost of dispersal may be manifested as increased mortality during dispersal (Johnson et al. 2009). Dispersal mortality is likely to be landscape-specific and may be elevated in certain situations (Schtickzelle et al. 2006). On the other hand, even though mortality would not be higher during dispersal, dispersing individuals inevitably lose time that could have been used for reproduction, thus adding to the cost of dispersal (Hanski et al. 2006). Movement, like all physical processes, requires energy and dispersing individuals need to pay an energetic cost. In some situations initiation of dispersal may require high enough body condition (Belthoff and Dufty 1998, Bonte and de la Pena 2009). Apart from the direct energetic costs, having the capacity for dispersal may lead to an additional trade-off cost if energy allocated to dispersal is drawn from sources that also supply other costly processes such as reproduction. There are several clear-cut demonstrations of such trade-off in wing-dimorphic species (Zera and Denno 1997). For example, it has been shown in the sand cricket (*Gryllus firmus*) that investing in flight muscles decreases early-life fecundity in females (Roff and Fairbairn 1991, Stirling et al. 2001) and testis size in males (Saglam et al. 2008).

High rate of energy consumption during dispersal could lead to faster senescence as increased energy expenditure may lead to elevated levels of oxidative damage (Costantini et al. 2008, Dowling and Simmons 2009). Energy expenditure and lifespan may thereby be negatively associated, as predicted by the 'rate of living—oxidative stress theory of aging' (Pearl 1928, Harman 1956, Prinzinger 2005) and evolutionary theories such as the 'disposable soma theory' (Kirkwood and Austad 2000, Hughes and Reynolds 2005). Such dispersal-lifespan trade-off could thus work against dispersal.

The process of dispersal is affected by many proximate factors (Bowler and Benton 2005). Dispersal, and emigration in particular, may be triggered by small area of habitat (Hill et al. 1996, Kuussaari et al. 1996, Crone et al. 2001) and to a certain extent by patch shape (Harper et al. 1993). This would happen if emigration is more likely when the likelihood of encountering the edge of the habitat is high, but small habitat patch size may also be correlated with other population-related parameters (Bowler and Benton 2005). The quality of the habitat matrix can affect dispersal (Wiens et al. 1993, Ricketts 2001, Berggren et al. 2002), especially in species that react strongly to habitat boundaries (Ries and Debinski 2001, Schultz and Crone 2001). Population density is often thought to be a key factor influencing emigration. Dispersal has indeed been show to be either positively (Shapiro 1970, Odendaal et al. 1989) or negatively (Kuussaari et al. 1996, Andreassen and Ims 2001) correlated with population density. Density dependence may be different between the two sexes (Baguette et al. 1998, Aars and Ims 2000, Loe et al. 2009). Positive density dependence could arise due to a high level of social interactions (Enfjäll and Leimar 2005) but also due to shortage of food resources (Schneider et al. 2003, Fred and Brommer 2009). In aphids the frequency of winged individuals and thus the tendency to disperse has been shown to increase with the presence of predators (Kunert et al. 2005, Mondor et al. 2005), parasitoids (Sloggett and Weisser 2002) and viruses (Ryabov et al. 2009), although the effect of predation may differ among aphid species (Kunert et al. 2008). Interactions among the kin, especially between mother and her offspring, may enhance dispersal as shown in e.g. lizards (Sinervo et al. 2006, Cote et al. 2007). On the other hand, in other species kin cooperation decreases dispersal (Lambin et al. 2001).

Dispersal may not be a random process with respect to the individuals that may disperse. In some organisms there exist distinct dispersive morphs, for instance individuals with substantial fat reserves in naked mole-rats (Oriain et al. 1996) and, perhaps most famously, long-winged, short-winged and wingless individuals in crickets, aphids and many other insects (Roff and Fairbairn 1991, Mondor et al. 2005). In other cases dispersive individuals lack obvious morphological or anatomical traits, but they may nonetheless exhibit more subtle differences. For instance, traits such as leg length in cane toads (Phillips et al. 2006) and relative thorax size in butterflies (Thomas et al. 1998, Hill et al. 1999, Hughes et al. 2003) have been linked to dispersal. Sedentary and dispersive individuals may besides not differ in appearance but differ in physiological performance (Haag et al. 2005). The dispersive phenotype may be under strong genetic control or affected more strongly by the environment (Roff and Fairbairn 1991, Zera and Denno 1997, Clobert et al. 2001).

Dispersing individuals may exhibit specialised behaviours (Van Dyck and Baguette 2005). Thus

butterflies flying within and outside their habitat may differ in their flight behaviour: flight tends to be faster and straighter in the latter habitat (Schultz 1998, Schtickzelle et al. 2007). Movements may be categorised as 'routine movements' used for everyday activities such as foraging and mate searching and 'special movements' aiming at dispersal (Van Dyck and Baguette 2005). Movement studies may be biased towards routine movements and thus potentially underestimate dispersal capacities. This bias is likely to depend on the difference of the scale of routine movements compared to the scale of dispersal events. When these two differ greatly, such as in the case of wing-dimorphic insects or spiders performing aerial dispersal by means of ballooning (Bonte et al. 2003), one may miss critical information on dispersal if only routine movements are studied.

1.3 Dispersal in fragmented landscapes as a model for studying natural selection

Anthropogenic habitat loss and fragmentation has become a global process and at the same time a major threat to biodiversity. Species that inhabit fragmented landscapes are dependent on dispersal for long-term persistence, the more so the smaller the average size of individual habitat fragments. Dispersal is thus especially important for the long-term persistence of classic metapopulations with no permanent (long-lasting) local populations. Dispersal connects different local populations into one metapopulation, and indeed with less dispersal local populations will become more isolated and may suffer from problems related to being small and isolated. Small population size contributes to high extinction risk for many reasons, including demographic stochasticity, Allee effects and inbreeding depression (Saccheri et al. 1998, Hanski 1999). In general, selection could therefore be expected to favour dispersal in fragmented landscapes. However, as dispersal may be costly, especially if the chance that a disperser will find suitable habitat is low, there are also factors that select against dispersal (Schtickzelle et al. 2006). The effect of habitat fragmentation on dispersal is therefore not a simple process, and models have shown that the evolutionary response may be either increased or reduced rate of dispersal depending on e.g. the current level of habitat fragmentation and other factors (Heino and Hanski 2001).

Opposing evolutionary forces may also act at the scale of local populations. The colonisation of a new population is not a random process, and every individual in the source population does not have an equal chance of establishing a new population. Individuals that establish new populations tend to be more dispersive than the population average (Hanski et al. 2002), and such higher mobility may be a heritable trait (Saastamoinen 2008). On the other hand, high average mobility (Hanski et al. 2004) or high proportion of winged individuals in wing-dimorphic species (Simmons and Thomas 2004) may start to decline rapidly following population establishment. The decrease in dispersal propensity is likely to be caused by two processes: loss of dispersive individuals through emigration, and local selection favouring less dispersive individuals via e.g. trade-off with fecundity (Olivieri et al. 1995, Hanski et al. 2004, Hanski and Saccheri 2006).

2 Aims of the thesis

The rapid development of molecular tools in recent years has made it possible to start bridging the gap between ecology and mechanistic studies of evolution. Studies on altitudinal adaptation in terms of variation in haemoglobin oxygen-binding affinity in deer mice (Storz et al. 2007), adaptive coat colour polymorphism in rock pocket mice (Hoekstra and Nachman 2003), and clinal variation in metabolic genes in wild fruit flies (Sezgin et al. 2004), to mention a few, have demonstrated the strength of studying evolution in the field.

There are often strong selection pressures affecting dispersal, and the relevant traits have high heritability (Roff and Fairbairn 1991, Saastamoinen 2008), which make dispersal an ideal target for studying natural selection in action. The aim of this thesis is examine the mechanistic basis of dispersal in the Glanville fritillary butterfly (*Melitaea* cinxia) to add to our understanding of the evolutionary processes operating in species living in fragmented landscapes. I link genetic variation to phenotypic variation, and genetic and phenotypic variation to organismal performance. I also test the association between genetic and phenotypic variation and one fitness measure. More specifically, I relate molecular variation in the gene phosphoglucose isomerase (*Pgi*) to flight metabolic rate and examine how molecular variation at *Pgi* and flight metabolic rate affect dispersal in the field. As an indirect fitness measure I use lifespan, which is correlated with lifetime reproductive output (Saastamoinen 2007b), and may be affected by metabolic rate.

The gene *Pgi* encodes the enzyme phosphoglucose isomerase, which has an important function in glycolysis. Previous work especially in *Colias* butterflies (see e.g. Watt et al. 2003) and the willow beetle (*Chrysomela aeneicollis*) (see e.g. Dahlhoff

and Rank 2000) has shown that this locus is highly polymorphic and that molecular variation in this gene has functional consequences. Although *Pgi* is known to affect metabolic fluxes, no systematic examination of the effect of variation in *Pgi* on whole-animal metabolic rate has been performed before (but see Kohane and Watt 1999, Haag et al. 2005, Dahlhoff et al. 2008). Measuring dispersal directly is challenging under field conditions. I have used mark-recapture methods but also a state-of-the-art technique, the scanning harmonic radar, which allowed collecting high-resolution data over large spatial scales for up to one day. Results on field-measured dispersal were further compared to results on tethered flight performed under controlled laboratory conditions. Lifespan was recorded in individuals kept under constant laboratory conditions, in the field, and under repeated exercise treatments.

This thesis addresses the following main questions:

- How is metabolic rate affected by factors such as body size, temperature and *Pgi* genotype? Do the same factors have similar effects on metabolic rate in different life-history and activity stages? (**I, II**)
- Is metabolic rate a repeatable trait, that is, a trait that has similar values for an individual in repeated measurements? (**IV**)
- Is resting metabolic rate correlated with flight metabolic rate? (**I**)
- To what extent can differences among individuals in movement activity and distance in the field be explained by metabolic rate, molecular variation at the *Pgi* locus and ambient environmental conditions? (**II, III**)
- Are the effects of metabolic rate and *Pgi* genotype on dispersal similar in the two sexes? (**III**)
- Is high metabolic rate associated with short lifespan? (**IV**)

3 The study species

The Glanville fritillary (*Melitaea cinxia*) is a north-temperate butterfly with a distribution ranging from South England to West China and from North Africa and Spain to central Scandinavia (Tolman and Levington 1997). In Finland the present-day distribution is limited to the Åland Islands situated between southwest Finland and Sweden. The species was previously more widely distributed in Finland with populations in the south-western part of the Finnish mainland, but the species has suffered from intensified agricultural practises and the mainland populations have gone extinct.

The Glanville fritillary is highly dependent on dry meadows with relatively low vegetation. The larvae use two host plants in Finland: the ribwort plantain (*Plantago lanceolata*) and the spiked speedwell (*Veronica spicata*). *Plantago lanceolata* occurs throughout the Åland Islands but *Veronica spicata* is most common in the western and northern parts of the archipelago. Female butterflies show oviposition preference for either host plant, and this preference is correlated with the regional abundances of the two plant species (Kuussaari et al. 2000).

The Glanville fritillary has a univoltine life cycle in the Åland Islands. In contrast, in the southern parts of its European distribution there are two or three generations per year (Kuussaari et al. 2004). Eggs are laid in clusters of 50 to 350 eggs on the underside of host plant leaves in June to early July. Oviposition typically occurs in the afternoon (Saastamoinen and Hanski 2008). The larvae hatch within a few weeks and feed gregariously. Larvae excrete a silky web that partially covers the host plants and the surroundings. Larvae shed their skin three or four times before entering winter diapause in early autumn. Larvae spend the winter in sibling groups inside a dense silk web structure. The size of the larval group has a positive effect on winter survival (Kuussaari et al. 2004). After snowmelt in late March or early April larvae terminate diapause and continue feeding and growing. After reaching the final instar the larval groups split up and larvae feed solitarily. Pupation takes place in early to mid May. The pupae hang among dense vegetation or rocks and are largely inconspicuous, although known to be attacked by pupal parasitoids (Lei et al. 1997). Emergence of adults occurs from the last week of May until mid June. The Glanville fritillary is a protandrous species with males emerging on average two to four days earlier than females (Boggs and Nieminen 2004). Male Glanville fritillaries typically exhibit perching behaviour when locating females (Wahlberg 2000), and thus they establish territories from which they perform rapid take-off flights to chase away intruding males and attempt to get access to females. The alternative mate locating strategy, patrolling, and intermediate forms also occur in the Glanville fritillary (Wahlberg 2000). Average lifetime movement distances have been found to be approximately 1500 m (Ovaskainen 2004), and newly-colonised populations rarely occur further than a few kilometres from the closest possible source populations (Hanski 1999). Adult

lifespan is from one to three weeks (Hanski et al. 2006, Saastamoinen et al. 2009).

The Glanville fritillary has been intensively studied in the Åland Islands since 1991. The population has been described as a classic metapopulation with discrete habitat patches, frequent extinctions of local populations and a high rate of re-colonisations of empty habitat patches (Hanski 1999). Different parts of the Åland metapopulation have been shown to follow largely independent dynamics (Hanski 1999). Demographic changes are monitored by surveying the study area twice a year. In the autumn, all potential habitat patches (ca 4,000) are being searched for the presence of larval groups, which are relatively easy to detect due to the conspicuous silken winter nests. In the following spring all populations found in the previous autumn are surveyed and the larvae are counted. The surveys also serve as an opportunity for collecting experimental individuals and samples for molecular studies with known population history.

4 Respirometry

The energy expenditure of an animal can be measured in several ways. Methods with which energy expenditure is measured directly are referred to as direct calorimetry. Direct measurements can be achieved by measuring the rate of energy release in the form of heat production, which is a direct consequence of energy use. In most situations, however, methods of indirect calorimetry are more applicable. One method with great potential in vertebrates is the use of doubly labelled water to assess field metabolic rate. The technique is based on the turnover rate of hydrogen and oxygen isotopes that have been injected in the study animals and quantified in two subsequent blood samples (Nagy 2005). Unfortunately, the doubly labelled water technique is not suitable for insects that rely on a tracheal oxygen transport system.

Insect metabolic rates are best measured as gas exchange rates. Simply, the amount of oxygen molecules (O_2) used is proportional to the amount of energy consumed, as is also the release of the end product of respiration, carbon dioxide (CO_2). The rate of CO_2 production depends, however, on the oxidised substrate. If the fuel is lipids, one molecule of oxygen consumed equals 0.7 molecules of CO_2 produced. When carbohydrates are oxidised the ratio is 1.0. The ratio between CO_2 produced and oxygen consumed is known as the respiratory quotient, *RQ*.

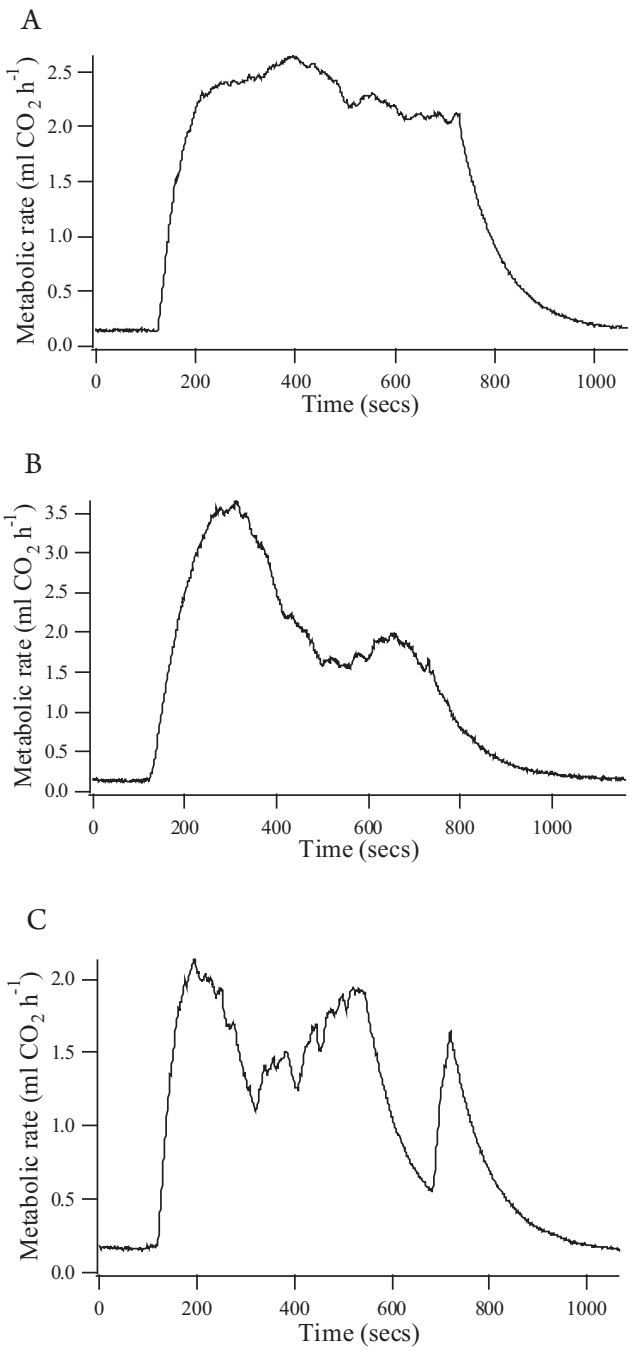

Fig. 1 Examples of 10-min measurements of flight metabolic rate. A) Female with continuous flight throughout the entire experiment. The mass of the individual was 94 mg and the total volume of CO_2 emitted in 10 mins was 0.429 ml. B) Male with high initial peak and continuous flight for 5 mins, after which it has flown in short bursts. The mass of the individual was 70 mg and the total volume of CO_2 emitted in 10 mins was 0.434 ml. C) Female with discontinuous flight throughout the experiment. The major dip in the CO_2 emission results from a 2-min period of no proper flight. The mass of the individual was 102 mg, and the total volume of CO_2 was 0.244 ml.

When the *RQ* lies between 0.7 and 1, both lipids and carbohydrates are used.

Measurement of oxygen consumption gives a very reliable estimate of metabolic rate. However, as the absolute changes in the oxygen concentration in air are minute when measuring small animals, measurements of oxygen consumption are considered problematic and error-prone in insects. When the *RQ* is known, whether based on careful measurements or biochemical knowledge of the oxidised resources, CO_2 production alone can be used as an estimate of metabolic rate. In the Glanville fritillary, the *RQ* during 15-min measurements of flight metabolism is very close to 1.0 (J.H. Marden, pers. comm.), indicating that carbohydrates are used as the source of energy designed for flight.

In the present studies metabolic rate was measured with an infrared absorption CO_2 analyser (Li-Cor 6251; Li-Cor Biosciences, Lincoln, NE, USA). The respirometry setup was based on the flow-through principle, in which the experimental animal is placed in a respirometry chamber with air flowing through the chamber at a known constant rate. The air entering the chamber has been scrubbed of CO_2, hence all the CO_2 detected by the analyser is being produced by the animal. The airflow was maintained with a pump (SS3 Subsampler; Sable Systems, Las Vegas, NV, USA) situated upflow from the respirometry chamber. Water vapour was removed from the air using Drierite (W.A. Hammond, Xenia, OH, USA) and CO_2 using first Medisorb (GE Healthcare, Chalfont St. Giles, UK) and then Ascarite II (Thomas, Swedesboro, NJ, USA). The air was dried again with magnesium perchlorate (Alfa Aesar, Karlsruhe, Germany) downstream of the respirometry chamber before entering the analyser. The temperature inside the respirometry chamber was recorded once a second using an NTC thermistor (Sable Systems). The data were gathered through a UI-2 interface (Sable Systems) and stored on a laptop computer with the software ExpeData (Sable Systems).

Metabolic rate was measured under three conditions: in flight (chapters I, II, III & IV), in resting adults (I &IV) and in pupae (I). These measurements differ in methodology and purpose. In the measurements of flight metabolic rate the experimental butterfly was flown inside a 1-L transparent respirometry chamber that was kept under a UV light source. The measurement temperature was controlled with an electric heater. No direct measurements of body temperature were taken but the body temperature should be very similar to the air temperature in the absence of other heat sources. Flight was induced by shaking or tapping the respirometer when the butterfly attempted to land on the walls of the chamber. In all flight experiments the measurement period was 10 minutes. The aim was to provoke the butterfly to fly as continuously as possible. There was significant variation among individuals, however, both in flight capacity and in flight endurance. Some individuals flew for the full 10-min period (Fig. 1a), while others flew well in the beginning of the experiment but showed more variable performance towards the end (Figs. 1b & c). Two traits were derived from these measurements, the peak flight metabolic rate and the total volume of CO_2 produced in 10 mins (flight metabolic rate). These two traits are highly correlated but the peak flight metabolic rate is a better indicator of the maximum metabolic capacity while the total volume of CO_2 describes flight endurance and has a stronger behavioural component.

Resting metabolic rate (RMR) is a very commonly recorded physiological variable. In endothermic vertebrates resting metabolic rate or the more strict measure of basal metabolic rate represent the minimum maintenance cost and are correlated with maximum metabolic rate (White and Seymour 2004). In insects the connections of the resting metabolic rate to other energetic or life history traits is less well established (Reinhold 1999, Niven and Scharlemann 2005). A great deal of the literature on insect resting metabolism focuses on the question of variable gas exchange patterns (Chown and Nicolson 2004). In short, insect respiration is based on a system of branching trachea and tracheoles through which oxygen moves directly to the mitochondria. Respiration is not based on passive diffusion only, as convection and active ventilation also play a significant role. Gas exchange can be continuous, cyclic or discontinuous and controlled by closing and opening of the spiracles that lead to the trachea. The exact purpose and origin of discontinuous gas exchange is still much debated, but it may be related to reducing respiratory water loss, be an adaptation to low-oxygen below-ground conditions or a mechanism to protect from oxidative damage (Lighton and Turner 2008, Schimpf et al. 2009). Alternatively, discontinuous gas exchange is not an adaptation, but rather a by-product of the nervous system controlling spiracular ventilation (Chown and Nicolson 2004). However, in the Glanville fritillary, resting metabolic rate is continuous (Fig. 2). There is short-term cycling in CO_2 production but no signs of clear bursts of CO_2 or full closing of spiracles. In chapters I and IV the resting metabolic rate is measured with the same setup as the flight metabolic rate. This setup with the 1-L respirometry

chamber yields an averaged value of the RMR because of the relatively slow washout of CO_2 in this measurement system in comparison with a setup designed for the measurement of RMR (Fig. 2). The advantage of the former setup however is that once the CO_2 emission curve has settled down to the resting level, even a short recording is sufficient for obtaining the measurement of RMR. Glanville fritillaries tend to be very passive indoors and remain practically immobile during the measurements when the respirometry chamber is covered. Rare occasions of activity in the chamber can be detected as a peak in the CO_2 level.

Pupal metabolic rate was measured with a customised setup consisting of a respirometry chamber of 7 ml and flushing air through the chamber at the rate of 480 ml min^{-1}. This system allowed detecting detailed patterns of gas exchange in the pupae. Pupae showed a response to handling in the beginning of the measurement but the metabolic rate stabilised after some minutes. The gas exchange pattern appeared continuous with some cycling (Fig. 3). In some cases pupae were found to close their spiracles for very short times, but the pattern was not consistent.

Metabolic rate scales positively with body mass (Kleiber 1947, Schmidt-Nielsen 1984). Metabolic rates therefore need to be corrected for differences in body mass when the intention is to compare individuals with various sizes. Metabolic rates are often expressed as mass-specific metabolic rates describing the metabolic intensity of a given amount

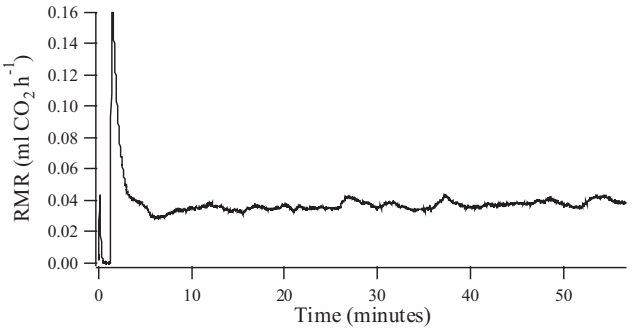

Fig. 2 Measurement of resting metabolic rate over a period of one hour. The measurement starts with recording a calibration gas with no CO_2, after which the flow is directed to the measurement chamber, which results in a burst of CO_2 that has been produced before the measurement. The resting metabolic rate appears continuous with no signs of discontinuous gas exchange or large cycling. In this measurement done at 23.8°C the chamber volume is 110 ml and the flow rate is set to180 ml min^{-1}.

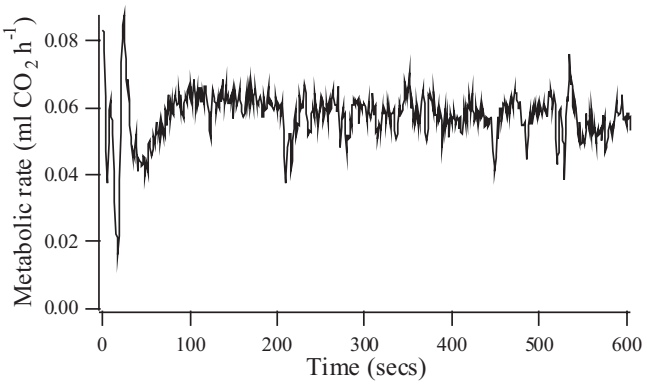

Fig. 3 Measurement of pupal metabolic rate. The setup allowed high-resolution measurements due to small chamber volume and high flow rate. Pupal metabolic rate appears continuous after the first few minutes.

of tissue. Typically this is achieved by dividing the metabolic rate by body mass. Metabolic intensity is not, however, mass-independent. As the slope for the relationship between log metabolic rate and log body mass is below 1, typically approximately 0.75, it follows that the mass-specific metabolic rate will correlate negatively with body mass with a slope of approximately -0.25. Therefore small individuals will have higher metabolic rates per given amount of tissue than larger individuals. The reasons for variation in metabolic intensity with body mass are not fully known (West et al. 1997, Kuikka 2003, Apol et al. 2008), but the pattern is nevertheless apparent both at the intraspecific and interspecific levels.

Size-independent metabolic rates can be calculated by means of linear regression (Hayes 2001). When the metabolic rate is regressed against body mass and residuals are extracted from the model, the residual metabolic rates reflect differences in metabolic rate that are fully independently of body mass. The drawback of using residual metabolic rates is that residuals from independent regressions are not comparable. Displaying uncorrected whole-animal metabolic rates along with body masses facilitates the comparison of different studies.

5 Harmonic radar

The scanning harmonic radar (Fig. 4) is suitable for tracking medium-sized insects, such as honeybees (Riley et al. 2005), bumble bees (Osborne et al. 1999, Riley et al. 1999), moths (Riley et al. 1998) and butterflies (Cant et al. 2005). Tracked individuals are equipped with a passive transponder that does not require an integrated energy source. The transponder is light-weighted (ca 7 mg) and consists of a 16 mm

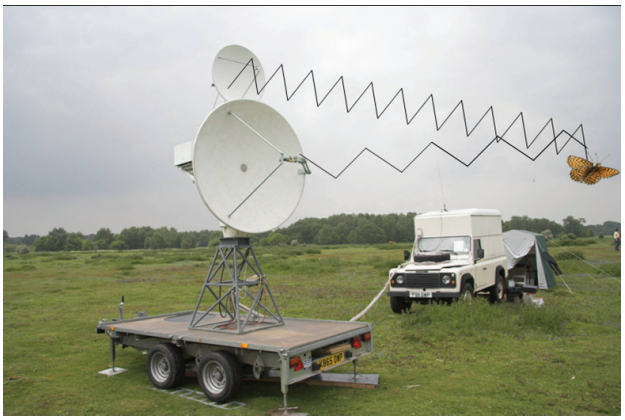

Fig. 4 The harmonic radar is suitable for tracking flying butterflies carrying a small transponder. The radar sends a signal that is altered by the transponder and received by the smaller radar disk that has been tuned to detect signals with a given wavelength. Photo by O. Ovaskainen.

half-wavelength dipole antenna with a diode in the middle. To ensure the stability of the transponder attachment, hairy cuticular scales are removed from the thorax and the surface is cleaned with alcohol after which a thin layer of glue is added. The transponder is attached on the thorax using double-sided sticky foam (Ovaskainen et al. 2008).

The harmonic radar can track individuals up to a distance of 900 m (Riley and Smith 2002). Unlike conventional radars, the harmonic radar does not detect all solid objects, but only the transponders. This is achieved by sending electromagnetic signals with a given wavelength (32 mm) and by tuning the receiver to detect signals with half the wavelength of the original signal (16 mm). When hit by the signal, the transponder reflects a signal at the second harmonic wavelength. The receiver of the radar then picks up the modified signal only and allows the detection of the spatial position of the individual with the transponder. The radar spins around a vertical axis at a speed of 20 revolutions per minute. An immobile transponder visible to the radar is thus detected every third second. Ovaskainen et al. (2008) examined the spatial accuracy of the radar data and calculated a mean radar error of 2.7 (±0.3) m.

While the harmonic radar is highly suitable for tracking free-flying insects there are also some limitations. First, the signals are not transported through solid objects, and therefore the study area must be free of obstacles such as trees and high bushes. The study area should also be flat, especially if the focal insects spend time close to the ground. Second, the radar does not distinguish individuals. The number of individuals that can be tracked in the same area is thus limited. Third, as individuals may be invisible to the radar when resting on the ground or among vegetation, the flight bouts should last longer than a few seconds. This is because the radar will only sweep through one particular area once in three seconds.

In the study presented in chapter II, the first of the above-mentioned problems was solved by selecting a distinctly flat area as the study site. The site, located in East Wretham, Norfolk, UK, consisted of nutrient-poor heathland. The vegetation on the heath was low due to grazing by sheep and rabbits and resembled the dry meadows occupied by the Glanville fritillary elsewhere. The larval host plant *Plantago lanceolata* was present at low densities in some parts of the heath. The second problem of the radar, namely not distinguishing multiple individuals, was solved by marking all butterflies with individual numbers on the hind wing. Three to six butterflies were released at the same time and scattered across the study site. The first release of each day was performed in the morning at approximately 11:00, and the second set of butterflies was released at approximately 14:00 in the afternoon. In the evening we aimed at collecting all individuals that still remained in the study area. Using this release and capture protocol we diminished the likelihood of having several butterflies in the same spatial location at the same time. However, when this occurred, the identities of butterflies could be confirmed by capturing the butterfly and reading the identity as written on the wing. The third problem, the limited detection capacity of the radar, made it impossible to detect most resting individuals but it did not usually affect the detection of flying individuals. The recorded flight bouts ranged from 7 to 414 m with a median length of 25 m (Ovaskainen et al. 2008). Therefore, despite its limitations, the scanning harmonic radar proved to be a useful means of tracking the flight of the Glanville fritillary in a natural environment.

6 *Pgi* and genotyping methods

The gene phosphoglucose isomerase (*Pgi*) encodes the glycolytic enzyme PGI. This gene and the corresponding enzyme has been the focus of much study for over 30 years starting from work by Ward B. Watt and colleagues (Watt 1977, Wang et al. 2009). The reason for the interest in *Pgi* is that it appears to be a gene under natural selection with significant single-gene effects on individual performance and fitness (Watt et al. 1983, Watt et al. 1985, Watt et al.

2003, Wheat et al. 2006). The effects of *Pgi* are best known in *Colias* butterflies (Pieridae). Differences in organismal performance have been traced to variation in enzyme kinetics and thermal stability (Watt 1983). In short, enzyme variants with high enzyme activity (V_{max}/k_m) are sensitive to temperature while variants with high thermal stability have low activities. The heterozygous enzyme variants, however, perform well over a broad range of temperatures and this effect is transferred to the organismal level (Watt and Dean 2000). Differences in biochemical properties can therefore have significant fitness effects and can even affect genotype frequencies among the butterflies that are active during the course of the day (Watt et al. 1983)—a phenomenon with great potential for sampling bias in field studies!

The *Pgi* polymorphism is not limited to *Colias* butterflies. Another successful model system is the willow beetle *Chrysomela aeneicollis*, which inhabits montane regions in the western part of North America. PGI allozyme alleles show clinal variation so that the thermally stable alleles are most abundant in the southern study populations and kinetically active alleles are most common in the northern populations (Dahlhoff and Rank 2000). PGI has also been linked to functional performance of the beetles, such as running speed (Rank et al. 2007), resting metabolic rate and reproductive output (Dahlhoff et al. 2008). The willow beetle studies have shown interactions between PGI genotypes and heat shock protein expression (Dahlhoff and Rank 2000). Interestingly, expression levels of the stress protein Hsp70 are highest in the thermally sensitive cool-climate specialist genotype, thus supporting the hypothesis about adaptations to thermally dissimilar environments (Neargarder et al. 2003, Dahlhoff and Rank 2007). Similar patterns of thermal adaptation indicated by variation in PGI allozymes and associated Hsp70 expression levels have also been reported from the copper butterfly *Lycaena tityrus* (Karl et al. 2008, 2009a, Karl et al. 2009b).

Studies on *Pgi* in the Glanville fritillary butterfly have revealed a high level of molecular variation with fitness consequences. Field-collected individuals from newly-established and old populations differ in their *Pgi* allele frequencies (Haag et al. 2005, Hanski and Saccheri 2006). Haag et al. (2005) showed that in female butterflies collected from the field there is a correlation between PGI (allozyme) genotype and flight metabolic rate and that flight metabolic rate also varied in relation to population age and spatial connectivity. Work by Saastamoinen demonstrated that *Pgi* variation is correlated with a whole suite of life history traits in the Glanville fritillary. The reproductive output of *Pgi-f* (*Pgi* SNP AA111 AC) females is significantly higher than that of *Pgi-non-f* (AA111 AA) females (Saastamoinen 2007a). These females have been found to have higher body temperatures in flight in low ambient temperatures (Saastamoinen and Hanski 2008), suggesting that this genotype is adapted to low temperatures. The genotype with high flight metabolic rate and high reproductive output has also been associated with long lifespan (Saastamoinen et al. 2009). In this thesis I study the effects of *Pgi* and interacting variables on flight metabolic rate (I & II). The effect of *Pgi* genotype on dispersal rate in the field is analysed in chapters II and III. In chapter IV the focus is on the links between *Pgi*, metabolic rate, and lifespan.

Work on PGI allozymes in the Glanville fritillary has revealed seven electromorph alleles in the Åland Islands, of which the two most common ones (d and f) account of 75% of all the allozyme copies (Saccheri et al. 1998, Haag et al. 2005). Sequencing the *Pgi* gene has revealed underlying DNA molecular variation, and the main allozyme alleles can be distinguished using single nucleotide polymorphisms (SNP) (Orsini et al. 2009). The allozyme allele f, which was found to be associated with high flight metabolic rate (Haag et al. 2005), is distinguished with the help of SNPs AA111 and AA361 located in the coding region of the *Pgi* gene. However, these two SNPs are highly linked and in most situations using the SNP AA111 alone is sufficient to tell apart the f allozyme genotype and to explain functional variation (Orsini et al. 2009). Thus individuals with the base pair combination AC at AA111 represent the f allozyme genotype as based on the allozyme analysis. Intriguingly, the CC homozygotes at AA111 are very rare in the Åland population, but the reasons for this are not clear (Orsini et al. 2009). The low frequency of CC homozygotes may be due to overdominance, linkage with some other deleterious locus or a combination of several factors. CC homozygotes are underrepresented also in a laboratory cross between heterozygous parents coming from the Åland Islands, whereas other populations are in Hardy-Weinberg equilibrium (Orsini et al. 2009).

In the work described in this thesis the SNP genotyping was performed using small wing samples from which DNA was extracted using a NucleoSpin tissue extraction kit (Macherey-Nagel, Düren, Germany). Megabase 1000 (GE Healthcare, Chalfont St. Giles, UK) was used for running primer extension reactions (SNuPe kit, GE Healthcare). Genotypes were called by SNP profiler (GE Healthcare) and checked manually. A detailed description of the genotyping process has been provided by Orsini et al. (2009)

7 Results and discussion

*7.1 Causes of variation in metabolic rate (**I & II**)*

Body mass had a consistent effect on metabolic rate in pupae, resting adults and flying adults. The effect of body size on metabolic rate is a solid fact that is consistent in practically all situations in intraspecific and interspecific comparisons. However, what the exact relationship is and what are the causes of the correlation are still much debated.

Temperature influenced metabolic rate in a different way in resting and flying individuals. In rest, metabolic rate increased between 2.1 and 2.6-fold with an increase of 10°C in temperature (Q_{10}) (calculated within the range of measurement temperatures from 29°C to 35°C and from 26°C to 32°C, respectively). These are very typical values for insects, where Q_{10} is usually between 2 and 3, depending on the temperature range in question (Chown and Nicolson 2004). In flight the effect of temperature depended on the ambient temperature and on *Pgi* genotype. In measurement temperatures below approximately 33°C *Pgi* AC individuals had consistently higher flight metabolic rate than *Pgi* AA homozygotes (Fig. 5). However, when the flight metabolic rate was measured at higher temperatures (up to 35°C) the pattern changed: the flight metabolic rate of *Pgi* AA individuals increased with temperature but that of AC heterozygotes began to decrease (Fig. 5). Thus *Pgi* AC individuals appear to be able to reach high flight metabolic rate already at lower temperatures but suffer from high temperatures, whereas AA homozygotes do relatively poorly at low temperatures but benefit of high temperatures. These results are consistent with the hypothesis that variants of the phosphoglucose isomerase enzyme differ in their kinetic properties and thermal sensitivity: isoforms with high kinetic values have low thermal stability, while isoforms with poor kinetics are often thermally stable (Watt 1977). Heterozygotes may then have superior kinetics and good thermal stability.

The effect of the time of the day on metabolic rate was significant in one experiment in chapter I, showing a nonlinear relationship with the highest rate in the early afternoon. This pattern was strongest in pupae but was present also in resting and flying adults. In other studies the effect of the time of the day has been negligible. The inconsistency in these results may be due to environmental conditions: in the study where the significant effect was found individuals were kept in an uninsulated building and not in a laboratory facility like in other studies. The effect may therefore be commonly present in nature but not in less variable, controlled laboratory conditions.

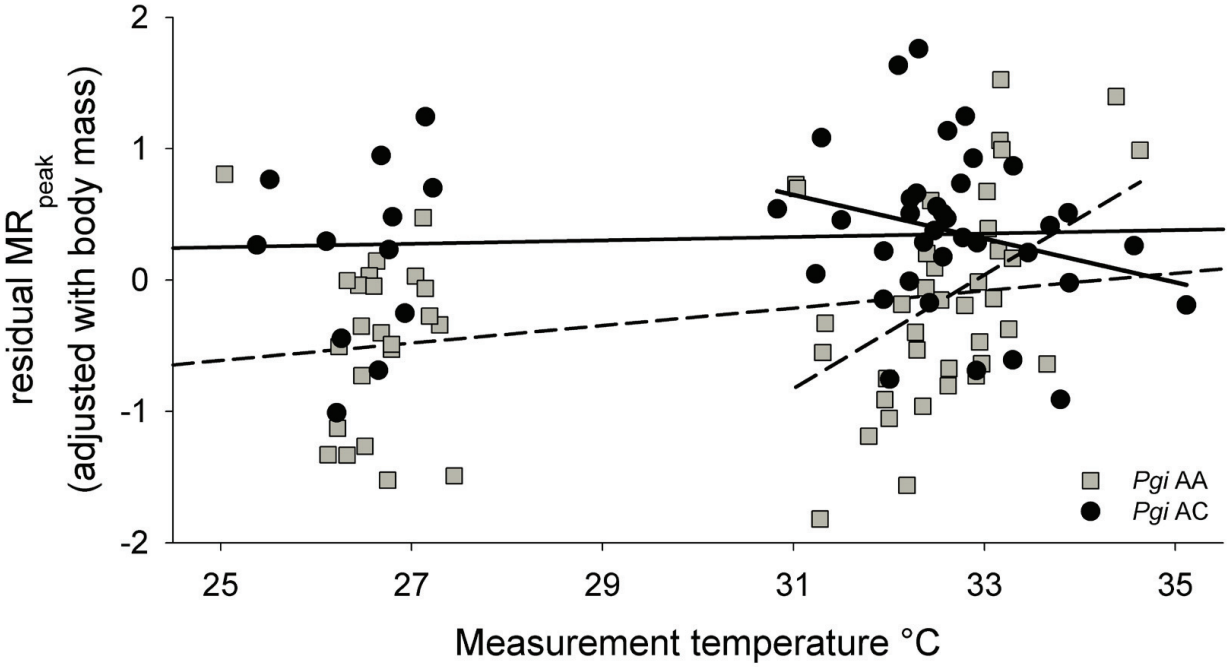

Fig. 5 Peak flight metabolic rate measured over a range of temperatures. The peak flight metabolic rate is consistently higher in *Pgi* AC individuals than in AA homozygotes over a range of temperatures, but at the highest temperatures the pattern is reversed (**Chapter I**).

Flight metabolic rate often exceeded resting metabolic rate several tenfold, reflecting the enormous demands on the metabolic machinery in flying insects. However, the difference between resting metabolic rate and peak flight metabolic rate, the factorial scope, was smaller than those reported for some other flying insects, typically exceeding 100 (Chown and Nicolson 2004). There are two likely reasons for this result. Firstly, the thermal conditions during the present measurements were set to optimal flight conditions. Thus resting metabolic rates were high due to their direct dependence on temperature. In classic studies such as the one by Bartholomew and Casey (1978), hawkmoths were measured at clearly lower temperatures ranging from 22 to 24°C. Endothermic hawkmoths are not capable of flight at such low body temperatures, hence they need to generate heat by muscle shivering, after which flight is possible (Heinrich 1993). Day-active butterflies such as the Glanville fritillary are functionally heliothermic, depending on direct radiation from the sun, and cannot fly at low body temperatures. In *Colias*, voluntary flight is known to begin at a thoracic temperature of 29°C (Watt 1968). Therefore the question of factorial scopes in ectothermic insects is somewhat arbitrary; the lowest resting metabolic rate is produced in conditions different from those suitable for maximum metabolic rate. Secondly, I suggest that flight may actually be relatively cheap in butterflies compared to many other flying insects. This is because butterflies have large wing areas, low wing loadings and low wing beat frequency, whereas insects such as hawkmoths and bees have narrower wings, higher wing loadings and higher wing beat frequency and thus more physiologically demanding flight.

No clear support for correlation between mass-independent resting metabolic rate and mass-independent peak flight metabolic rate was found. The relationship was examined in two datasets, of which the first one contained no pattern suggestive of a relationship between the two traits. In the second dataset a significant correlation was found, but the proportion of variation explained was so low that it could be concluded that resting metabolic rate has very little predictive value on peak flight metabolic rate. Also in all other datasets examined (K. Niitepõld, unpublished data), no correlation has been found. The resting metabolic rates in the second dataset were relatively high, probably reflecting high initial physiological activity of butterflies, which may be due to conditions in which the butterflies were maintained. The finding of no clear correlation between resting metabolic rate and maximum metabolic rate differs from the consistent correlation that is found in vertebrates.

7.2 Pgi, *physiology and dispersal (**II & III**)*

Flight metabolic rate was found to have a clear effect on dispersal rate in females that were tracked with harmonic radar (Fig. 6). Variation in the distance moved in 1 h was almost 100-fold among females, but one third of this variation could be explained by flight metabolic rate. *Pgi* genotype was found to influence flight metabolic rate and movement rate in a similar way: in low to moderate temperatures *Pgi* AC heterozygotes had higher flight metabolic rate and moved longer distances in the field than AA homozygotes. Thus in environmental conditions that are typical for North Europe, AC heterozygotes are better fliers and more likely to disperse than AA homozygotes. These mechanistic findings match perfectly the observed pattern that individuals from newly-established local populations are more mobile than individuals from old populations (Hanski et al. 2002, Hanski et al. 2004, Hanski et al. 2006) and that the frequency of AC heterozygotes is highest in isolated newly-established populations (Haag et al. 2005). When taking into account the significant heritability of mobility in females (Saastamoinen 2008), it seems clear that mobile females are more likely to disperse and establish new local populations than less mobile females.

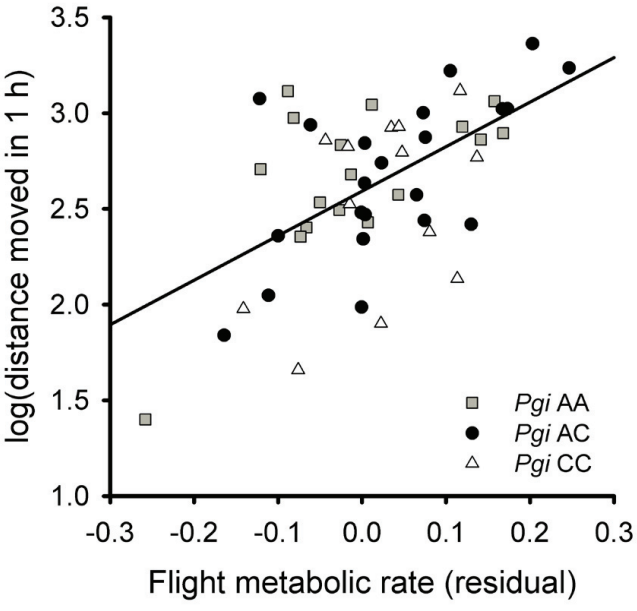

Fig. 6 Dispersal rate measured with harmonic radar was affected by flight metabolic rate (**Chapter II**).

Dispersal is clearly a complex and multi-stage process (Bowler and Benton 2005, Van Dyck and Baguette 2005), including components such as dispersal willingness and dispersal capacity (Yukilevich 2005) and condition-dependent decisions (Clobert et al. 2009). Not every flight event outside the habitat patch is directly connected to dispersal, as in the case of looping flights commonly observed in many butterflies (Schtickzelle and Baguette 2003, Conradt and Roper 2006, Crone and Schultz 2008). The results of chapter II may have caught some of the complexity of dispersal, as the 5-min mobility of females with the *Pgi* AA genotype was found to be lower in individuals with high flight metabolic rate. In contrast, in AC heterozygotes the correlation was positive, as was also found in the analysis of 1-h mobility.

Chapter III examines the genetic and physiological traits affecting dispersal but now comparing the two sexes. The evolution of dispersal may be apparently different in males and females as investment in reproduction differs between the males and females and flight is known to have partly different functions in the two sexes. The results presented in chapter III show that males and females indeed differ in terms of the relationship between flight physiology and dispersal. Peak flight metabolic rate correlated significantly and negatively with dispersal distance in males that were followed with mark-recapture methods. This result may be due to mate locating behaviour. Male Glanville fritillaries establish territories and show aggressive behaviour towards other males. Males with high peak flight metabolic rate may therefore be successful territory holders with no need to move long distances at the landscape scale. Males with less agile flight and low peak flight metabolic rate would consequently be unsuccessful in gaining territories and would be forced to disperse more. Flight behaviour was also tested in tethered conditions and there males with the longest flight durations had low peak flight metabolic rates. This result suggests that at least some of the males with low peak flight metabolic rate could nonetheless sustain long flight duration, as would be advantageous for an individual with the patroller strategy. The mate location strategy of males has been shown to correlate with flight performance-related morphology in the speckled wood butterfly (*Pararge aegeria*) in which perchers have relatively larger thoraces than patrollers (Van Dyck et al. 1997, Berwaerts et al. 2002). Peak flight metabolic rate could act as a similar measure of flight ability that is adapted to certain behavioural strategies.

In females the mark-recapture study did not yield any significant correlation between peak flight metabolic rate and dispersal distance. This contradicts earlier results and there is no simple reason for the lack of correlation in this particular study. The experimental setup may be one reason, as the study was conducted on a small island, which may have restricted the maximum movement distances. However, when examining long distance dispersers separately from individuals that were only observed in their release patch or very close to it, a significant interaction between peak flight metabolic rate and sex was found when explaining residual dispersal rate. This analysis did show a positive association between peak flight metabolic rate and time-corrected dispersal distance in females. Moreover, in the tethered flight experiment there was a positive relationship between peak flight metabolic rate and flight duration in females, suggesting that flight metabolic rate and flight behaviour are intimately related in females.

Dispersal was also studied in relation to *Pgi* genotype. Here, too, a contrasting relationship between the genotype and dispersal was found between the two sexes. Males with the *Pgi* AA genotype were more mobile than AC heterozygotes, while in females the pattern was the opposite. Unfortunately, this study suffered from low sample size and the results were statistically nonsignificant.

*7.3 Consequences of high metabolic rates (**IV**)*

Life history theory emphasises the importance of trade-offs between various fitness components. It is therefore reasonable to ask whether high flight metabolic rate would carry a cost affecting fitness. A natural candidate fitness component that could be expected to be affected by high energy expenditure is lifespan, as suggested by the rate of living theory (Pearl 1928). Chapter IV demonstrates that there indeed is a significant relationship between peak flight metabolic rate and lifespan, but the correlation is positive, not negative. Resting metabolic rate does not correlate with lifespan, and certainly not in a negative manner. Chapter IV also examines the repeatability of peak flight metabolic rate over the course of the lifespan. Peak flight metabolic rate was found to be a significantly repeatable trait. Flight metabolism was affected by senescence only at the very end of the lifespan; peak flight metabolic rate fell to a very low level 0 to 2 days before the death.

Consistent patterns in energy expenditure throughout the lifespan and positive correlation between flight metabolism and lifespan clearly indicate that simple hypotheses drawn from the rate of living theory do not explain variation in lifespan in the Glanville fritillary. Instead, individuals seem

to differ in their physiological condition that is positively correlated with many fitness components. This finding is in line with previous results for the Glanville fritillary showing that *Pgi* AC heterozygotes have higher fecundity (Saastamoinen 2007a) and longer lifespan (Saastamoinen et al. 2009) than *Pgi* AA homozygotes.

Ageing and lifespan are complex traits, and other studies have shown that the often assumed interspecific negative relationship between mass-specific resting metabolic rate and lifespan may be an artefact of body size (Speakman 2005, Furness and Speakman 2008). At the intraspecific level, like in chapter IV, several studies have reported no relationship or a positive relationship between energy expenditure and lifespan (Oklejewicz and Daan 2002, Melvin et al. 2007). The mechanistic basis of the 'oxidative stress theory' has also been challenged, as the production of reactive oxygen species (ROS) has been shown to be very low at high cellular respiration rate (Kowaltowski et al. 2009).

8 Conclusions and prospects

Dispersal has critical fitness consequences and is often under strong selection, especially in fragmented landscapes (Heino and Hanski 2001, Ronce 2007). The mechanistic basis of dispersal is therefore an interesting model system for learning more about evolutionary processes in the wild. The interesting question is how molecular variation translates into functional variation in free-ranging individuals. Previously genomic tools were available only for model species that could be studied in laboratory environments, but as the molecular and genomics methods are developing a growing number of studies is focusing on variation in natural populations.

This thesis links genetic variation to phenotypic variation and examines how genotype and phenotype influence performance in the field. The candidate gene *Pgi* is found to affect flight metabolic rate, dispersal rate, and to be associated with lifespan. *Pgi* is found to interact with temperature, suggesting adaptation to the prevailing thermal conditions.

Metabolic rate, and the flight metabolic rate in particular, is the central trait of these studies. Flight metabolic rate is a measure of flight ability and was found to correlate positively with dispersal rate measured with harmonic radar in female Glanville fritillaries. Flight metabolic rate has a strong genetic component, reflected by the association with molecular variation in *Pgi*, but is also a plastic trait, partly due to an interaction between *Pgi* genotype and temperature. Moreover, flight metabolic rate is affected by body size. Direct measurements of mobility in the field corroborate previous indirect findings: dispersal is a non-random process, and certain genotypes and phenotypes are more likely to disperse and potentially establish new populations.

The two sexes were found to differ in their relationship between flight metabolic rate and dispersal. In fact, males with high peak flight metabolic rate moved less at the landscape scale than males with low peak flight metabolic rate. This could be due to behavioural differences between males and females acting under different evolutionary pressures; while flight in females may serve the purpose of long-distance dispersal, one of the primary functions of flight in males is mate locating. Males with high peak flight metabolic rate may invest in establishing territories for gaining access to females, and males with lower flight ability and low competitive success would be forced to disperse. Low peak flight metabolic rate can also be an adaptation to continuous flight in males that patrol in search of females. For future studies, it would be useful to study the relationship between metabolic rate and male behaviour in more detail and to investigate also other species, in which the alternative mate locating strategies 'perching' and 'patrolling' are clearly distinguishable. Also, the consistency of mate locating strategies in changing conditions would be worth exploring.

Genetic polymorphism and variation in dispersal rate may be maintained by frequent colonisation events in metapopulations inhabiting fragmented landscapes. Interestingly, the mobile and metabolically active phenotype seems to be adapted to cool temperatures, which is in line with predictions from enzyme kinetics (Watt et al. 1983) as well as findings from certain *Drosophila* mutants that prefer low temperatures and possess elevated metabolic rate (Takeuchi et al. 2009). These results have some interesting consequences when considered in the context of responses to climate change. Many butterflies have been documented to have shifted their distributions northwards at a very rapid rate (Parmesan et al. 1999, Pöyry et al. 2009). It has been suggested that populations at the northern range margin would be preadapted to warming, but experimental evidence for this hypothesis has been mixed (Hellmann et al. 2008, Pelini et al. 2009). In contrast, results presented here would suggest that marginal populations would have a higher proportion of mobile and thus cool-adapted individuals. Traditionally dispersal is seen as a force acting against local adaptation (Kawecki 2008), but in the case of some butterflies the process of dispersal could select for individuals that are preadapted to

suboptimal local conditions at the northern (or upper) range of the distribution. The existence of dispersive phenotypes can increase range expansion speed (Thomas et al. 2001, Phillips et al. 2006) but here it could also increase fitness after settlement due to correlated life history traits. Nevertheless, species with specialised ecologies or restricted host plant distributions are facing serious challenges in changing climatic conditions.

The relationship between peak flight metabolic rate and lifespan was clearly positive, and peak flight metabolic rate remained relatively constant with age. Resting metabolic rate was not related to lifespan. These findings refute the hypothesis, based on the rate of living theory, that high energy expenditure should lead to short lifespan. Therefore, the physiological cost of flight capacity appears negligible in the Glanville fritillary. The positive correlation between peak flight metabolic rate and lifespan was observed also in individuals that spent most of their lives in the field, and in individuals that were repeatedly forced to fly. It remains to be seen whether increasing the number and/or duration of flight treatments would produce a similar effect and if some genotypes and phenotypes would be more tolerant to increased flight activity. The results indicate that variation in an individual's physiological condition could be a critical factor in determining the lifespan of individuals, and that there are significant positive relationships among various fitness traits. Ageing is a complex process that does not always follow attractive but simple hypotheses (Hulbert et al. 2007, Furness and Speakman 2008).

9 Acknowledgements

I thank my supervisor Ilkka for all the support and guidance that I have received during my time in the Metapopulation Research Group. Ilkka has been the ideal supervisor by allowing me plenty of freedom in planning and designing experiments and in developing ideas. When help is needed—and it often has—Ilkka is always reachable be it morning, evening, weekend or holiday in Lapland. Everyone from master's students to professors who has worked with Ilkka has presumably been impressed by Ilkka's swift replies to emails and his amazing skills in editing manuscripts. Working with Ilkka has been highly inspiring and I have certainly learned a lot. Thank you, Ilkka.

Jim Marden was never an official supervisor of mine, but Jim has been very much involved in my work and deserves my warmest gratitude. Jim showed me how to perform respirometry on butterflies and trusted his equipment in my hands for all these years. I have warm memories starting from the pioneering experiment at Nåtö biological station that basically laid the foundation for my thesis. I also thank Jim for his hospitality during my visit to Penn State.

I am most grateful to the people at Rothamsted Research with whom I had the privilege to work with. Rebecca Nesbit and Jason Chapman were incredibly helpful when setting up and carrying out the tethered flight experiment. Thank you also for organising activities such as pancake feasts and birding trips at a time when especially Jason was having busy days with a new baby in the house. The harmonic radar experiment together with Juliet Osborne, Alan Smith, Andrew Martin, Don Reynolds and Norman Carreck was unforgettable from the start and will be remembered for a long time. It was very inspiring to follow your team in action and I admire your ability and determination to overcome all the challenges that appeared during the course of the field work. It was a pleasure to work with you.

I am in great debt to Marjo Saastamoinen for all the help she has given to me during these years. My first appointment with the MRG was when I served as Marjo's field assistant in her cage experiment back in 2004. During that time it became clear to me that I undoubtedly wanted to do a PhD in the group. Discussions with Marjo have served a major role in developing my research and it has been very useful and pleasant to have such a brilliant 'academic older sister'. We survived the allozyme scoring in Tvärminne!

I thank my co-authors Otso Ovaskainen, Phil Harrison and Anniina Mattila. My former neighbour Otso did a magnificent job in squeezing the relevant parameters out of the raw, noisy radar data. Besides, now I know whom to turn to when I need custom generated data sets. It has been great to learn to know Phil who is one of the most devoted and hardworking persons I have met, and fun to hang around with. Later duder. At the time of writing this, Anniina was granted funding from the Luova graduate school for her PhD work. It has been rewarding to work with you in Åland and in the laboratory and I wish you all the best for the future.

I have had a number of wonderful office mates during my time in the MRG. Heidi Viljanen and Helena Wirta (then Koivulehto) were great company when we all had just started our PhD studies. Anna-Liisa Laine moved in for a short while before moving on to the Luova coordinator office. My longest-standing offices mates, Chris Wheat and Ayco Tack deserve a very special thank you. It has been very valuable to be able to ask stupid questions about genes and genomics or mixed models and random factors, or to have the occasional discussion on politics and society or philosophy and religion. It has been great fun. After Chris (temporarily) left Helsinki Éva Tas joined our office team and fit in perfectly. The only thing I am worried of is how anyone will manage to switch on the right lights after I am gone.

I am grateful to all the students taking part in the cinxia surveys and collecting larvae from Åland. I thank all the people assisting with the rearing of larvae and participating in carrying out the experiments. My special thanks go to Suvi Ikonen for her expertise on practical butterfly work and for successfully running the impressive rearing facilities at Lammi biological station.

I thank Toshka Nyman and Luisa Orsini for help and guidance in the laboratory when genotyping my samples. Your assistance was invaluable and I definitely learned a lot. (In most cases the hard way.)

We here in the MRG are lucky to enjoy the fantastic service from our wonderful secretaries. Some of you have already left, some will still return and some have left and returned and left again but still will come back! Thanks for everything, you are the most important people, Mimma, Nina, Eveliina, Tuuli, Sami and Marika.

I want to thank everyone in the MRG. Working with you has been stimulating, and the concept of a group of great people with diverse backgrounds working on various subjects functions very well. There has always been somebody to ask from when help is needed. Members of the MRG have been excellent company during conference trips and also outside work. During the last months the practical and mental support from Helena, Heidi, Jenni and Varpu has been of immense importance, as we all will defend our theses within a period of a few months.

I thank Janne Kotiaho and Bas Zwaan for graciously performing the pre-examination of my thesis and for giving insightful comments.

I am grateful to my parents for giving me the opportunity to grow up in a home where curiosity, asking questions and discussing various matters was encouraged. I thank you for all the support and help throughout these years. Thank you for the time spent in the countryside in Häme in Finland and on the island of Orust in Sweden. Much of my interest for nature stems from having been able to stroll in the forests of Riisikkala and on the rocky outcrops of Örnviken. Kiitos äiti, aitäh isa. I thank my sisters Sirje and Merike for being great company; it has always been fun to spend time with you, at home, in the car, or travelling abroad.

I thank my dear wife Inka for support and patience. Thanks for keeping things going even when my mind has still been at work. Your help has been much appreciated.

Finally, I thank our lovely daughter Aime for bringing so much love and joy to my life. You are such an amazing person, full of spirit and always ready for new adventures. KIITOS AIME.

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